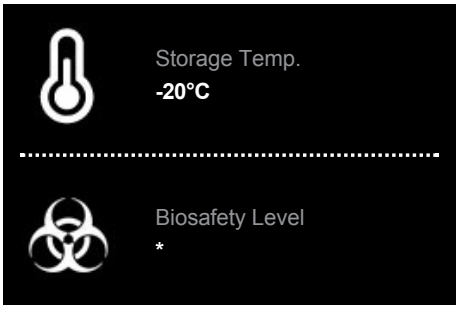
ATCC

Product Sheet

Trypsin-EDTA for Primary Cells (ATCC® PCS-999-003™)

Please read this FIRST



Description

Product Description:

Trypsin-EDTA for Primary Cells is a low-concentration formulation (compared to ATCC ® 30-2101) of porcine pancreatic trypsin and EDTA that is suitable for the dissociation of cell monolayers that are susceptible to "over-trypsinization." These adherent cells include primary cells (i.e., ATCC® Primary Cells Solutions™ cell types) as well as a variety of mammalian cell lines that are propagated in serum-free or low serum conditions.

Formulation: 0.05% Trypsin, 0.02% EDTA in phosphate buffered saline without calcium or magnesium.

Additional Reagents Needed for Subculture:

1. DPBS (ATCC® 30-2200)
2. Trypsin Neutralizing Solution (ATCC® PCS-999-004)

Does not contain phenol red.
Volume: 100 mL

Directions for Use

General Subculture Procedure

Each type of cell or cell line responds to Trypsin-EDTA for Primary Cells in a unique manner. For optimum results, continuously observe the cells during the dissociation process to prevent damage. For cell-specific information, please refer to the product sheet supplied with the cells or cell line.

1. Bring the DPBS, the Trypsin-EDTA for Primary Cells, and the Trypsin Neutralizing Solution to room temperature before use. Warm the complete growth medium to 37°C prior to use with the cells.
2. For each flask, carefully aspirate the spent media without disturbing the monolayer. If the cell culture medium contains serum, each flask should be rinsed with DPBS twice prior to adding the Trypsin-EDTA for Primary Cells.
3. Using 1 to 2 mL for every 25 cm2, add the appropriate volume of trypsin-EDTA solution to each flask (e.g., each T-25 flask would be dissociated with 1 to 2 mL trypsin-EDTA).
4. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells, and then aspirate the excess fluid off of the monolayer; do not aspirate to dryness.
5. Observe the cells under the microscope. When the cells pull away from each other and round up (typically within about 3 to 6 minutes), remove the flask from the microscope and gently tap the culture flask from several sides to promote detachment of the cells from the flask. Do not over-trypsinize as this will damage the cells.
    a. Some strongly adherent cell types, such as keratinocytes, may take much longer and may require trypsinization at 37°C.
    b. Some cell types may require more vigorous tapping.
6. When the majority of cells appear to have detached, quickly add an equal volume of the Trypsin Neutralizing Solution to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
7. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the culture flask.
8. Add 3 to 5 mL DPBS to the tissue culture flask to collect any additional cells that might have been left behind.
9. Transfer the cell / DPBS suspension to the centrifuge tube containing the trypsin-EDTA-dissociated cells.
10. Repeat steps 8 and 9 as needed until all cells have been collected from all flasks.
11. Centrifuge the cells at 150 x g for 3 to 5 minutes.
    a. Do not over centrifuge cells as this may cause cell damage.
    b. After centrifugation, the cells should form a clean loose pellet.
12. Aspirate neutralized dissociation solution and resuspend the cell pellet in 2 to 8 mL fresh, pre-warmed, complete growth medium.
13. Count the cells and seed new culture flasks at the recommended density.
14. Place newly seeded flasks in a 37°C, 5% CO_2 incubator and incubate for at least 24 to 48 hours before processing the cells further.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium

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Or contact your local distributor

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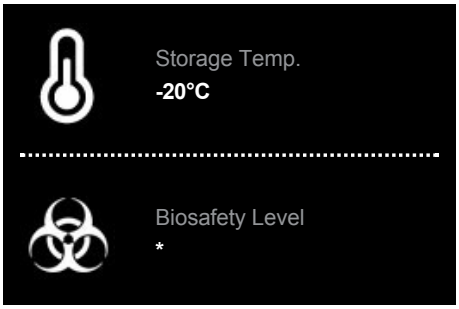

formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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